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Dietary cholesterol and cardiovascular disease: a systematic review and meta-analysis[1–3]

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ABSTRACT

Background: Dietary cholesterol has been suggested to increase the risk of cardiovascular disease (CVD), which has led to US recommendations to reduce cholesterol intake.

Objective: The authors examine the effects of dietary cholesterol on CVD risk in healthy adults by using systematic review and meta-analysis.

Design: MEDLINE, Cochrane Central, and Commonwealth Agricultural Bureau Abstracts databases were searched through December 2013 for prospective studies that quantified dietary cholesterol. Investigators independently screened citations and verified extracted data on study and participant characteristics, outcomes, and quality. Random-effect models meta-analysis was used when at least 3 studies reported the same CVD outcome.

Results: Forty studies (17 cohorts in 19 publications with 361,923 subjects and 19 trials in 21 publications with 632 subjects) published between 1979 and 2013 were eligible for review. Dietary cholesterol was not statistically significantly associated with any coronary artery disease (4 cohorts; no summary RR), ischemic stroke (4 cohorts; summary RR: 1.13; 95% CI: 0.99, 1.28), or hemorrhagic stroke (3 cohorts; summary RR: 1.09; 95% CI: 0.79, 1.50). Dietary cholesterol statistically significantly increased both serum total cholesterol (17 trials; net change: 11.2 mg/dL; 95% CI: 6.4, 15.9) and low-density lipoprotein (LDL) cholesterol (14 trials; net change: 6.7 mg/dL; 95% CI: 1.7, 11.7 mg/dL). Increases in LDL cholesterol were no longer statistically significant when intervention doses exceeded 900 mg/d. Dietary cholesterol also statistically significantly increased serum high-density lipoprotein cholesterol (13 trials; net change: 3.2 mg/dL; 95% CI: 0.9, 9.7 mg/dL) and the LDL to high-density lipoprotein ratio (5 trials; net change: 0.2; 95% CI: 0.0, 0.3). Dietary cholesterol did not statistically significantly change serum triglycerides or very-low-density lipoprotein concentrations.

Conclusion: Reviewed studies were heterogeneous and lacked the methodologic rigor to draw any conclusions regarding the effects of dietary cholesterol on CVD risk. Carefully adjusted and well-conducted cohort studies would be useful to identify the relative effects of dietary cholesterol on CVD risk. *Am J Clin Nutr* 2015;102:276–94.

Keywords: dietary cholesterol, cardiovascular disease, serum cholesterol, HDL cholesterol, LDL cholesterol

INTRODUCTION

Dietary cholesterol has been suggested to increase the risk of cardiovascular disease (CVD),[6] which led to the 2010 Dietary Guidelines Advisory Committee recommendations of no more than 300 mg/d of cholesterol for healthy populations in the United States (1, 2). The International Atherosclerosis Society also recommends decreasing dietary cholesterol as a strategy for lowering LDL cholesterol (3). The 2010 dietary cholesterol recommendations were derived from guidelines established in the 1960s, when little scientific evidence was present, other than the possible association between saturated fat and dietary cholesterol, as well as animal studies, in which cholesterol was fed in amounts that exceeded typical or normal intakes (4). In contrast to dietary guidelines in the United States, other developed and developing countries do not have an upper limit on cholesterol intake but focus on controlling the intake of saturated and *trans* fat, which are major determinants of blood cholesterol concentrations (5).

Since the US dietary cholesterol recommendations were first promulgated, a large number of longitudinal observational studies and intervention trials have been published on the relation between cholesterol intake and cardiovascular outcomes. Some studies have reported dietary cholesterol to increase CVD risk (6–10), whereas others reported a decreased risk or no change with higher cholesterol intake (11–13). Given these inconsistencies among studies, the purpose of this systematic review is to determine the potential effect of dietary cholesterol on incident CVD and on serum concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, LDL to HDL ratio, and triglycerides in adults.

METHODS

Overview

This study is a systematic review of the literature on the effects of dietary cholesterol on CVD risk. We held meetings

[1] Supported by USDA agreement 1950-51000-073 and the American Egg Board, Egg Nutrition Center.

[2] The funders did not have a role in the study selection, quality assessment, data synthesis, or manuscript preparation.

[3] Supplemental Tables 1–10 and Supplemental Figures 1–14 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[6] Abbreviations used: CAD, coronary artery disease; CVD, cardiovascular disease; TEP, technical expert panel.

Received September 30, 2014. Accepted for publication May 7, 2015.

First published online June 24, 2015; doi: 10.3945/ajcn.114.100305.

Supplemental Material can be found at:
http://ajcn.nutrition.org/content/suppl/2015/06/24/ajcn.114.100305.DCSupplemental.html

and teleconferences with a technical expert panel (TEP) that served as a group of scientific partners who identified issues central to this topic and provided methodologic and subject matter expertise. The TEP comprised a nutritional epidemiologist specializing in CVD, a cardiologist, a statistical methodologist with expertise in meta-analyses, and a research dietitian. See **Supplemental Table 1** for a list of TEP members and affiliations. The TEP worked with the authors to refine key questions, identify important issues, and define eligibility criteria for the review. A standard protocol was developed and followed for the overall review. A single causal pathway, or analytic framework, was developed (**Supplemental Figure 1**). Study results were reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (14).

Data sources and study eligibility

We conducted a comprehensive literature search from 1946 through December 2013 for publications on dietary cholesterol in adult humans indexed in MEDLINE, Cochrane Central, and Commonwealth Agricultural Bureau Abstracts. Searches were not restricted by using study design filters. We also cross-referenced the citations from relevant systematic reviews and citations of recovered articles. The searches combined terms for dietary cholesterol, CVD, and serum lipids (**Supplemental Table 2**). The literature searches were screened in duplicate by using study eligibility criteria, and discrepancies were resolved by consensus in group conference.

Study inclusion criteria

We included prospective cohort and intervention studies in adults ≥18 y of age that quantified the amount of cholesterol intake per day or per week from any dietary source. Cardiovascular-related outcomes of interest included coronary heart disease, ischemic heart disease, coronary artery disease (CAD), angina, myocardial infarction, heart failure, hypertensive heart disease, cerebrovascular disease (ischemic stroke and hemorrhagic stroke), peripheral artery disease, CVD death, and serum lipids (total cholesterol, LDL, HDL, LDL/HDL, VLDL, and triglycerides). Only English-language publications were included.

Study exclusion criteria

We excluded cross-sectional, retrospective cohort, case-control, and single-arm studies (interventions with no control group). Studies in which participants had major chronic diseases such as cancer, diagnosis of CVD, or chronic kidney disease at baseline were excluded. We also excluded studies in children, pregnant women, and any trials using a weight loss or lifestyle modification program. Interventions with an unbalanced dietary fat (amount or type of fatty acids) between intervention and control arms were excluded. We also excluded studies that did not assess the association of cholesterol intake with relevant outcomes of interest.

In addition to the above common eligibility criteria, we established the following additional criteria specific to study design.

Cohort studies

In our analysis of cohort studies, we included studies that recruited participants without CVD diagnosis at baseline. Studies were eligible if the population was healthy or if CVD risk factors, including hypertension, hyperlipidemia, metabolic syndrome, or diabetes, were present at baseline. Included studies had at least one quartile category or study group with cholesterol intake >300 mg/d. Studies that reported multivariable results adjusting for any potential confounders were deemed eligible. No minimum study duration or follow-up time was required for inclusion.

Intervention trials

In analysis of intervention trials, we included studies that recruited participants with normal/healthy lipid concentrations at baseline and excluded studies in individuals with hyperlipidemia, metabolic syndrome, or diabetes at baseline. We also excluded studies in participants that used cholesterol-lowering drugs or statin therapy. The minimum duration for all blood lipid studies was 4 wk, and we excluded studies with <5 subjects per arm.

Data extraction and quality assessment

Data from each study were extracted independently by one of 4 investigators and confirmed by at least one other. The extracted data included study design; participant characteristics; longest reported follow-up period; method of assessing dietary cholesterol intake or details of dietary cholesterol intervention; association between dietary cholesterol intake or intervention and outcome; potential confounding variables adjusted for, with particular emphasis on age, sex, weight, smoking, and variables related to other dietary exposure (e.g., fiber, carbohydrate, and fats); method of ascertaining CVD and lipid outcome; and statistical analyses.

We assessed the methodologic quality of each study based on predefined criteria, in accordance with the Agency for Healthcare Research and Quality–suggested methods for systematic reviews (15). Study quality was determined in duplicate, and discrepancies were resolved by consensus in group conference. Good-quality (low risk of bias) studies adhere most closely to the commonly held concepts of high quality, including clear descriptions of the population and setting; unbiased assessments of dietary cholesterol status and outcomes; appropriate statistical analysis, including multivariable analysis adjusting for age, race, weight, and dietary fats; no obvious reporting omissions or errors; and <20% dropouts. Fair-quality (medium risk of bias) studies have some deficiencies in the above criteria unlikely to cause major bias. Poor-quality (high risk of bias) studies have major deficiencies such that major bias could not be excluded. We considered factors in the modified Newcastle-Ottawa Scale (16) for observational studies, the Cochrane risk of bias for clinical trials (17), and nutrition-specific items from a critical appraisal of micronutrient systematic reviews for both clinical trials and observational studies (18).

Data synthesis

We performed random-effects model meta-analyses when similar data from 3 or more observational cohorts or trials were available (19). For observational studies, we synthesized RRs (or

HRs or ORs) comparing the extreme categories of dietary cholesterol (highest compared with lowest, as defined within each study) provided that the categories corresponded to similar doses of cholesterol intake across studies. When the metrics for exposure or outcome measure varied across cohorts, the studies were not combined. For example, studies reporting the effect of dietary cholesterol on disease risk per 100-mg increase in dietary cholesterol were not combined with studies reporting risk between lowest and highest quartiles of cholesterol intake in milligrams per day. In addition, we did not combine studies when the dose in the lowest quartile of one study was similar to the dose in the highest quartile of another study.

For intervention studies, we combined net differences [Net change = (Dietary cholesterol$_{Final}$ − Dietary cholesterol$_{Initial}$) − (Control$_{Final}$ − Control$_{Initial}$)] for continuous outcomes. We tested between-study heterogeneity with the Q statistic (significant when $P < 0.10$) and quantified its extent with I^2 (20). We collected reported data for the LDL to HDL ratio as reported in study results; therefore, the LDL to HDL ratio values were not manually calculated by using other serum cholesterol results. When randomized trials performed post hoc subgroup analysis that broke study randomization, we grouped the post hoc data with data from other nonrandomized trials.

Subgroup and dose-response analyses

To explore potential reasons for differences of results across studies and to evaluate possible dose effects, we performed several meta-regression analyses with continuous dietary cholesterol dose (mg/d) and serum lipid concentrations (mg/dL). Random-effects meta-regression was performed to assess the impact of variables on net change in serum lipids. Dose-response curves were determined by using the PROC NLIN function in SAS version 9.3 (SAS Institute). When appropriate, we performed subgroup meta-analyses, including age and sex. We evaluated the potential for publication bias with funnel plots and Egger's tests for small study effects. Analyses were performed in STATA version 13 (StataCorp LP) with the metan, metareg, and metabias functions.

RESULTS

The searches identified 7107 abstracts. After title and abstract screening, 543 articles were retrieved for full-text review, and 40 studies met eligibility criteria. An outline of the evidence search and study inclusion is provided in **Supplemental Figure 2**. A detailed list of exclusion reasons is provided in **Supplemental Table 3**. The most prevalent reasons for article exclusion were "no dietary cholesterol reported" (n = 140) and "comparison not relevant" (n = 135).

Cohort studies

We included 17 prospective cohort studies [in 19 publications (6, 9, 13, 21–36); **Table 1**]. Ten studies examined CAD (21–23, 25–29, 32, 35), 6 studies examined stroke (9, 13, 24, 30, 31, 36), and one study examined both CAD and stroke outcomes (6). These studies were conducted in the United States, United Kingdom, Japan, Puerto Rico, and Sweden. Most of the studies compared quantile categories of baseline cholesterol intake per day by using food-frequency questionnaires or 24-h dietary recalls. Six studies estimated the association of CVD outcomes and baseline cholesterol intake in milligrams per kilocalorie of energy intake (6, 21, 23, 25, 27, 32). Studies reporting any CAD, CAD death, nonfatal CAD, or any CVD reported varying exposure and/or outcome metrics, precluding meta-analysis. Details about inclusion and exclusion criteria (**Supplemental Table 4**) and baseline diet information for individual cohort studies (**Supplemental Table 5**) can be found in the online supplemental material. The methodologic quality of the cohort studies was graded good or fair, reflecting low or medium risk of bias, respectively (**Supplemental Table 6**).

Any CAD (fatal or nonfatal)

Four cohort studies [The Puerto Rico Heart Health Program (23), Honolulu Heart Study (27), Strong Heart Study (35), and Framingham Study (29)] reported the association between baseline cholesterol intake and risk of incident fatal or nonfatal CAD (Table 1). The studies included 19,057 participants who were followed from 6 to 16 y. Three studies included all men (23, 27, 29), and one study included both men and women (35). Each study included participants with a different race or ethnicity: Caucasians (29), American Indians (35), Japanese (27), and Puerto Ricans (23). All studies assessed cholesterol intake by self-reported data at baseline, and only one assessed intake data at second examination (35). All studies were rated fair quality (23, 27, 29, 35). Ascertainment of CAD was by examination of multiple records in one (35) but was not reported in others. Only one study adjusted for dietary variables of protein and energy (35), and none adjusted for dietary fat, a major factor affecting the quality of the studies.

There was no association between higher cholesterol intake and an increased risk of incident CAD in 2 studies (29, 35). Three studies reported the association between cholesterol intake in milligrams per kilocalories and risk of incident CAD (23, 27, 29). Higher cholesterol intake (mg/kcal) was statistically significantly associated with incident CAD in men of Japanese ancestry in one study (27), but no similar association was found in Caucasian (29) or Puerto Rican men (23).

CAD death

Seven cohort studies (in 9 publications)—The Health Professionals Follow-Up Study (21), Lipid Research Clinics Prevalence Study (22), Ireland-Boston Diet Heart Study (25), Mann et al. (26), Finnish Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (28), Western Electric Study (32–34), and Strong Heart Study (35)—reported the association between dietary cholesterol and risk of death secondary to CAD (**Supplemental Figure 3**). All except 2 studies were conducted in North America (26, 28). The studies included 86,798 participants who were followed from 6 to 25 y. Four studies included all men (21, 25, 28, 32), and 3 studies included both men and women (22, 26, 35). Only one study reported information on race data (100% American Indians) (35). All studies assessed dietary cholesterol intake by self-reported data at baseline, and only 2 studies assessed intake data at second examination (32, 35). One was rated good quality (21), 5 were rated fair quality (22, 26, 28, 32, 35), and one study was rated poor quality because of participant dropout >30% (25). Ascertainment of CAD death was by examination of death certificates (25, 26, 28, 32),

TABLE 1
Characteristics of cohort studies examining the association between dietary cholesterol and cardiovascular disease[1]

Lead author, year: cohort (country) (ref)	Mean age (range), y	Number analyzed (% male)	Dietary cholesterol[2] (tool)	Outcome[3] (follow-up)	Baseline comorbidities	Maximum adjusted covariates
Larsson, 2012: Swedish Mammography Cohort (Sweden) (9)	62[4] (49–83)	34,670 (0)	302 vs. 168 (FFQ)	Cerebral infarction; hemorrhagic stroke; total stroke (10.4 y)	3% with DM; 20% with HTN	Age, smoking, education, BMI, physical activity, history of HTN, history of DM, aspirin, family, history of MI, alcohol, protein, fiber, total fat, saturated fat, MUFA, PUFA, ALA, **ω**-3, and **ω**-6 PUFA
Yaemsiri, 2012: Women's Health Initiative (United States) (36)	64 (50–79)	87,025 (0)	311 vs. 133 (FFQ)	Ischemic stroke (7.6 y)	Small percentage of the following: DM, CAD, HTN, and medication use	Age, race, education, family income, smoking, DM, HRT, total metabolic equivalent task hours, alcohol intake, history of CAD, history of atrial fibrillation, aspirin use, antihypertensive medication use, cholesterol-lowering medication use, BMI, SBP, total energy intake, dietary vitamin E, fruit and vegetable intake, and fiber
Houston, 2011: Health ABC Study (United States) (6)	75 (70–79)	1941 (45)	147 vs. 67 mg/1000 kcal (FFQ)	Incident CVD (9 y)	15–20% DM; 49–55% HTN; 9–14% statin use; 27–35% aspirin use; mean serum cholesterol = 208 mg/dL	Age, sex, race, education, field center, smoking, alcohol use, physical activity, BMI, total energy, protein, fiber intake, multivitamin use, supplemental vitamin E use, statin use, aspirin use, oral estrogen use (women only), prevalent DM or HTN, saturated fat, MUFA, PUFA, and *trans* fat
Xu, 2006: Strong Heart Study (United States) (35)	60[4] (47–79)	2938 (39)[4]	607 vs. 83 (24-h recall)	Any CAD; nonfatal CAD (7.2 y)	49%[4] with HTN	Sex, age, study center, DM, BMI, HDL, LDL, TAG, smoking, alcohol, HTN, % of energy from protein, and total energy intake
Sauvaget, 2004: Adult Health Study (Japan) (30)	57 (35–89)	3731 (39)	624 vs. 152 (24-h diary)	Death from cerebral infarction (14 y)	Radiation doses from atomic bomb exposure; 36% history of HTN; 29.3% current smokers; 7% DM	Age, sex, radiation dose, city, BMI, smoking status, alcohol, medical history of HTN and DM, fruit and vegetable intake, animal protein, and animal fat

(Continued)

TABLE 1 (*Continued*)

Lead author, year: cohort (country) (ref)	Mean age (range), y	Number analyzed (% male)	Dietary cholesterol[2] (tool)	Outcome[3] (follow-up)	Baseline comorbidities	Maximum adjusted covariates
He, 2003: Health Professionals Follow-Up Study (United States) (24)	NR (40–75)	43,732 (100)	398 vs. 189 (FFQ)	Ischemic stroke; hemorrhagic stroke (14 y)	None	BMI, physical activity, history of HTN, smoking, aspirin use, multivitamin use, alcohol, potassium, fiber, vitamin E, total fruit and vegetables, total energy intake, hypercholesterolemia at baseline, PUFA, MUFA, saturated fat, and *trans* fat
Iso, 2001: Nurses' Health Study (United States) (13)	46 (NR)	85,764 (0)	465 vs. 212 (FFQ)	Total stroke; ischemic stroke; subarachnoid hemorrhage (14 y)	7% with alcohol intake ≥25 g/d, some with hormone therapy use, multivitamin use, vigorous exercise, and some with HTN or DM	Age; smoking; time interval; BMI; alcohol use; ω-3, menopausal status; postmenopausal HRT; vigorous exercise; usual aspirin; multivitamins; vitamin E; fatty acid intake; calcium intake; histories of HTN, DM, and high cholesterol; and total energy intake
Mann, 1997: NR (United Kingdom) (26)	39[4] (NR)	10,802 (38)[4]	Men: 431 vs. 156; women: 378 vs. 138 (FFQ)	Death from CAD (13.3 y)	20% current smokers; 46% any smoking; 20% with BMI >24 kg/m^2	Age, sex, smoking, and social class
Ascherio, 1996: Health Professionals Follow-Up Study (United States) (21)	NR (40–75)	43,757 (100)	422 vs. 189 (FFQ)	Total MI (6 y)	18–20% with HTN; 6–14% current smokers; serum cholesterol = 201–205 mg/dL	Age, BMI, smoking, alcohol, physical activity, history of HTN, high blood cholesterol, family history of MI before age 60 y, profession, fiber, energy intake, and total fat
Esrey, 1996: Lipids Research Clinic Prevalence Follow-up Study (United States and Canada) (22)	57[4] (30–79)	4546 (59)[4]	Age 30–59 y: 427 vs. 416; age 60–79 y: 423 vs. 355 (24-h recall)	Death from CAD (12.4 y)	None	Age, sex, energy intake, serum lipids, SBP, cigarette smoking status, BMI, and glucose intolerance

(Continued)

TABLE 1 (*Continued*)

Lead author, year: cohort (country) (ref)	Mean age (range), y	Number analyzed (% male)	Dietary cholesterol[2] (tool)	Outcome[3] (follow-up)	Baseline comorbidities	Maximum adjusted covariates
Posner, 1991: Framingham Heart Study (United States) (29)	56 (45–65)	813 (100)	300 vs. ≥530 (24-h recall)	Incident CAD; death from CAD (16 y)	Around 40% were smokers	Energy intake, physical activity, serum cholesterol concentration, SBP, LV hypertrophy, cigarette smoking, glucose intolerance, and Metropolitan Life Insurance Company relative weight
Shekelle, 1981, 1989; Stamler, 1993: Western Electric Study (United States) (32–34)	NR (40–55)	1824[5] (100)	289–500 vs. 81–185 (Burke)[6]	Death from CVD; death from CAD (19–25 y)	High mean serum cholesterol of 247.7 mg/dL	Age, SBP, smoking, serum cholesterol, alcohol, BMI, and parents' country of birth
Kushi, 1985: Ireland-Boston Diet Heart Study (United States and Ireland) (25)	NR (NR)	1001 (100)	831 (FFQ)	Death from CAD (18 y)	Blood pressure, body weight, and smoking were higher in Boston than Ireland brothers	Age, SBP, total serum cholesterol, smoking, alcohol, and cohort of origin
McGee, 1984: Honolulu Heart Program (United States) (27)	NR (45–68)	7088 (100)	553 (24-h recall)	Total CAD; death from MI or CAD (10 y)	None	Age, SBP, serum cholesterol, cigarettes smoked, body weight, and physical activity
Garcia-Palmieri, 1980: Puerto Rico Heart Health (Puerto Rico) (23)	NR (45–64)	8218 (100)	Urban: 439; rural: 356 (24-h recall)	Incident CAD; death from MI and CAD (6 y)	None	Carbohydrates, alcohol, SBP, serum cholesterol, smoking, and blood glucose

[1]ALA, α-linolenic acid; CAD, coronary artery disease; CVD, cardiovascular disease; DM, diabetes mellitus; FFQ, food-frequency questionnaire; Health ABC, Health, Aging and Body Composition; HTN, hypertension; HRT, hormone replacement therapy; LV, left ventricular; MI, myocardial infarction; NR, not reported; ref, reference; SBP, systolic blood pressure; TAG, triacylglycerol.

[2]Dietary cholesterol expressed as mg/d unless otherwise specified.

[3]The primary outcome. If the primary outcome is not defined, then the outcomes that were meta-analyzed.

[4]Calculated mean from subgroups.

[5]Sample size from the Shekelle and Stamler (33) article.

[6]Burke method = detailed interview about usual eating, 3-d diet record, and FFQ.

from medical records (35), or both (21, 22). All studies reported multivariable adjusted results; only 2 studies adjusted for dietary variables (21, 35), and only one adjusted for dietary fat (21).

The association between higher cholesterol intake and an increased risk of incident CAD death was not statistically significant in 3 studies (21, 28, 32). In one study that recruited both men and women, there was a statistically significant increase in CAD death in the highest tertile category for men (median intake of 431 mg/d) and mid-tertile category for women (median intake of 245 mg/d) (35). Of note, this study included more women than men (62%) and a higher number of vegetarians or vegans than omnivores (42%).

Three studies reported the association between cholesterol intake in milligrams per kilocalories and risk of incident CAD death (21, 32). Higher cholesterol intake (mg/kcal) was statistically significantly associated with death from CAD in one study at 19 y (32), but no similar association was found in the other 2 studies (21, 25).

Nonfatal CAD

Four studies reported the association between baseline cholesterol intake and risk of incident nonfatal CAD: The Health Professionals Follow-Up Study (21), Puerto Rico Heart Health Program (23), Honolulu Heart Study (27), and Strong Heart Study (35) (Table 1). The studies included 62,814 participants who were followed from 6 to 16 y. Three studies included all men (21, 23, 27), and one study included both men and women (35). All studies assessed cholesterol intake by self-reported data at baseline, and only one assessed intake data at second examination (35). All studies were rated fair quality. Ascertainment of CAD was clearly defined in 2 (21, 35) of 4 studies (21, 23, 27, 35). Only one study adjusted for dietary variables of protein and energy (35), and one adjusted for dietary fiber, energy, and fat (21).

Three studies showed no association between higher cholesterol intake and an increased risk of incident nonfatal CAD across studies (21, 23, 35). One study (27) found a higher risk of CAD with a higher intake of dietary cholesterol (mg/1000 kcal).

Any stroke

Two studies—Nurses' Health Study (13) and Swedish Mammography Cohort (9)—reported the association between baseline cholesterol intake and risk of any stroke (Table 1). The studies included 120,434 women participants who were followed from 10 to 14 y. Both studies assessed cholesterol intake by self-reported data at baseline. One was rated good quality (9) and the other fair quality (13). Ascertainment of stroke was reported through national registry data (9) or by examination of multiple records (13). Both studies adjusted for dietary variables, and only one adjusted for dietary fat (9). We did not perform meta-analyses because there were only 2 available studies.

The Nurses' Health Study (13) found no significant association between higher intake of cholesterol and incidence of any stroke. A statistically significant increased risk of stroke was observed for cholesterol intake in quantile categories and as a continuous variable in the Swedish Mammography Cohort (9).

Subtypes of stroke

Of the 6 cohort studies, 5 [Health Professionals Follow-Up Study (24), Nurses' Health Study (13), Shibata Study (31), Swedish Mammography Cohort (9), and Women's Health Initiative–Observational Study (36)] reported the association between baseline cholesterol intake and risk of any ischemic stroke, and one study [Adult Health Study (30)] reported on fatal stroke (Table 1). The studies included 257,205 men and women participants who were followed from 7 to 14 y. All studies assessed cholesterol intake by self-reported data at baseline, and one study assessed by repeated measurements (24). Three were rated good quality (9, 24, 31), and the remaining 3 studies were rated fair quality (13, 30, 36). Ascertainment of stroke was reported through national registry data (9), through death certificates (30), or by examination of multiple records (13, 24, 31, 36). All 6 studies adjusted for dietary variables; only 3 studies adjusted for dietary fat (9, 24, 31).

Ischemic stroke

In meta-analysis of 5 studies of ischemic stroke (**Figure 1**), no statistical significance was found with the highest quantile categories (summary adjusted RR: 1.13; 95% CI: 0.99, 1.28) without any heterogeneity (I^2 = 0.0%; P = 0.42). There was no statistically significant increased risk of ischemic stroke and increasing doses of cholesterol intake characterized as milligrams per day (as a continuous variable) in the Swedish Mammography Cohort (9).

Hemorrhagic stroke

In meta-analysis of 3 studies of hemorrhagic stroke (9, 13, 24), no statistical significance was found with the highest quantile categories (summary adjusted RR: 1.09; 95% CI: 0.79, 1.50) without any heterogeneity (I^2 = 0.0%; P = 0.82) (Figure 1).

Any CVD

One study, the Health ABC Study (6), examined the association between intake of dietary cholesterol and any CVD, including CAD, stroke, and cardiovascular-related death. There was no statistically significant increased risk of CVD with increased cholesterol intake characterized as milligrams per day (as a continuous variable).

Intervention studies

Nineteen unique trials among 21 publications (8, 37–56) reported outcome data on dietary cholesterol and serum cholesterol concentration. Seventeen of the 19 unique trials (8, 37–53, 56) reported quantitative data and were included in meta-analysis (**Table 2**). Two studies with results that could not be combined with other studies were not included in the meta-analysis. One study did not have data available to calculate final SD (55), and one study reported total cholesterol increases but did not provide baseline, SD, or statistical data (54). Study duration of the 17 trials included in the meta-analysis ranged from 4 to 12 wk. The intervention cholesterol dose ranged from 501 to 1415 mg/d, and the control cholesterol dose ranged from 0 to 415 mg/d. All except 4 trials were conducted in North America (8, 38, 49, 56). All 17 intervention studies reported the effect of dietary cholesterol on changes in total serum cholesterol. Of the 17 trials, 15 reported the effect of dietary cholesterol on serum LDL cholesterol concentration (8, 37, 38, 40, 43–52, 56), 14 reported the effect of dietary cholesterol on

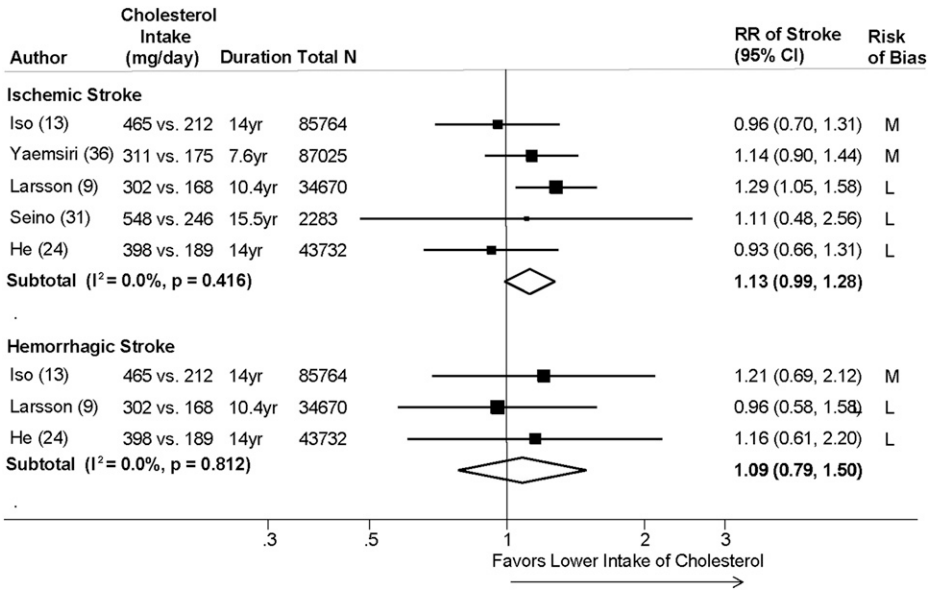

FIGURE 1 Meta-analysis examining the association between dietary cholesterol and ischemic and hemorrhagic stroke. Cholesterol intake corresponds to highest and lowest quartiles. Black squares represent point estimates for individual studies, and the width of the line extending from each square represents the 95% CI. The diamond represents the overall effect, and the width of the diamond represents the 95% CI of the summary relative risk. I^2 is an indicator of between-comparison heterogeneity. Heterogeneity was significant at $P < 0.10$. L, low risk of bias; M, medium risk of bias.

HDL cholesterol (8, 37, 38, 40, 42–48, 50, 51, 56), 13 reported the effect of dietary cholesterol on triglycerides (8, 37–46, 48, 56), and 3 studies reported the effect of dietary cholesterol on VLDL cholesterol (38, 40, 56). Further details about inclusion and exclusion criteria (**Supplemental Table 7**) and baseline diet information for individual intervention studies (**Supplemental Table 8**) can be found in the online supplemental material. The methodologic quality of the intervention studies was graded good or fair, reflecting low or medium risk of bias, respectively (**Supplemental Tables 9–10**). A summary of overall effects across a meta-analysis of all serum cholesterol outcomes can be found in **Table 3**.

Total cholesterol

Meta-analysis of 18 trials (8, 37–52, 56) reporting the effect of dietary cholesterol on serum total cholesterol showed a significant increase in serum total cholesterol when comparing intervention with control doses of dietary cholesterol (net change: 11.2 mg/dL; 95% CI: 6.4, 15.9) but with significant heterogeneity ($I^2 = 75\%$, $P < 0.001$). When stratified by randomization design, the change in serum total cholesterol concentration remained statistically significantly increased in randomized trials (net change: 7.4 mg/dL; 95% CI: 1.8, 12.9) and in nonrandomized trials (net change: 18.1 mg/dL; 95% CI: 9.5, 26.7) but with significant heterogeneity ($I^2 = 75\%$, $P < 0.001$, and $I^2 = 66\%$, $P < 0.003$, respectively). Among the 13 randomized trials, 8 were rated with a low risk of bias (37, 38, 43–45, 49, 52, 56). All nonrandomized trials were assigned a medium risk of bias (8, 39, 41, 42, 46).

When studies were stratified by intervention dose of dietary cholesterol, studies reporting an intervention dose <650 mg/d and studies with an intervention dose between 650 and 900 mg/d were statistically significant [net change: 12.1 (95% CI: 6.0, 18.2) and net change: 10.7 mg/dL (95% CI: 5.4, 15.9), respectively; Table 3 and **Supplemental Figure 4**]. In studies with an intervention dose >900 mg/d, the change in serum total cholesterol was no longer statistically significant (13.0 mg/dL; 95% CI: −3.1, 29.1) with significant heterogeneity ($I^2 = 94\%$, $P < 0.001$). When extreme dose values (intervention doses >1000 mg/d and control doses <1 mg/d) were removed from the meta-analysis, studies with intervention doses >900 mg/d showed a significant increase in total cholesterol concentration (net change: 19.3 mg/dL; 95% CI: 2.3, 36.3; **Figure 2**) with significant heterogeneity ($I^2 = 75\%$, $P = 0.02$). When nonrandomized studies were removed from analysis, the effect on serum total cholesterol did not change.

Additional analyses were performed to separate studies by the control dose of dietary cholesterol. When studies were separated by control doses >200 mg/d and ≤200 mg/d, the effect on total cholesterol remained significant in both strata [net change: 11.6 (95% CI: 2.0, 21.0) and net change: 11.1 (95% CI: 6.8, 15.4), respectively]. Heterogeneity was significant in studies with control doses of cholesterol >200 mg/d ($I^2 = 83\%$, $P < 0.001$) but not in studies with control doses ≤200 mg/d ($I^2 = 42\%$, $P = 0.06$).

LDL cholesterol

Of the 15 trials that reported the effect of dietary cholesterol interventions on serum LDL cholesterol (8, 37, 38, 40, 43–52, 56), 13 were randomized (37, 38, 40, 43–45, 47–52, 56) and 2 were nonrandomized trials (8, 46).

Meta-analysis of trials investigating LDL cholesterol showed a significant increase in LDL cholesterol when comparing intervention with control doses of dietary cholesterol (net change: 6.7 mg/dL; 95% CI: 1.7, 11.7; Table 3) but showed significant heterogeneity ($I^2 = 65\%$, $P < 0.001$). When stratified by randomization, the net change in serum LDL cholesterol concentration was significant in randomized studies but not in nonrandomized studies. Among the randomized trials, the net

TABLE 2
Characteristics of intervention studies examining the association between dietary cholesterol and blood cholesterol concentrations[1]

Lead author, year (country) (ref)	Study design	Mean age (range), y	Number analyzed (% male)	Dietary cholesterol dose[2] (difference)	Mean ± SD baseline TC, mg/dL	Intervention description	Outcomes (duration)
Mutungi, 2008 and 2010 (United States) (50, 53)	RCT (parallel)	NR (40–70)	28 (100)	827 vs. 277 (550)	High dose: 198 ± 42; low dose: 188 ± 33	Subjects consumed either 3 liquid eggs per day or the same volume of egg substitute for 12 wk. Subjects were free-living, and their diet was not restricted or supplemented outside the intervention.	TC, LDL, HDL, LDL to HDL (12 wk)
Nissinen, 2008 (Finland) (8)	nRCT (crossover)	54 (NR)	29 (100)	890 vs. 200 (690)	232 ± 8	Subjects consumed a low-cholesterol, low-fat diet; a high-cholesterol, low-fat diet; and a low-cholesterol, high-fat diet, each for 6 wk with at least 3 mo washout between periods.	TC, LDL, HDL, TG (6 wk)
Greene, 2005 (United States) (45)	RCT (crossover)	NR (NR)	42 (31)	892 vs. 267 (625)	Men: 197 ± 29; women: 207 ± 48	Subjects were instructed to avoid egg consumption and maintain regular diets. Subjects also received either egg or egg substitute.	TC, LDL, HDL, TG, LDL to HDL (4 wk)
Herron, 2003 (United States) (46)	RCT (crossover)	33[3] (20–50)	40 (100)	821 vs. 183 (638)	Hyperresponders: 157 ± 29; hyporesponders: 154 ± 33	All subjects adhered to the NCEP step 1 diet, and subjects consumed liquid eggs or egg substitute. Subjects were instructed to consume >300 mg cholesterol/d.	TC, LDL, HDL, TG, LDL to HDL (4 wk)
Herron, 2002 (United States) (47)	RCT (crossover)	30[3] (18–49)	10 (0)	640 vs. 0 (640)	Caucasian: 178 ± 31; Hispanic: 169 ± 27	All subjects adhered to the NCEP step 1 diet. Subjects were assigned to 3 eggs per day or egg substitute for 30 d followed by a 3-wk washout period followed by 30-d crossover to the other intervention.	TC, LDL, HDL, LDL to HDL(4 wk)
Reaven, 2001 (United States) (52)	RCT (parallel)	56 (NR)	65 (0)	941 vs. 113 (828)	176 ± 6	Subjects were assigned a 4-wk baseline diet of 113 mg/d, a 4-wk washout period, and 4-wk of a diet containing 319 mg, 523 mg, or 941 mg/d. Eggs and egg substitute were used to alter the cholesterol content, and all other macronutrients remained constant across diets.	TC, LDL (4 wk)
Duane, 1999[4] (United States) (55)	RCT (crossover)	60[3] (43–80)	84 (100)	505 vs. 23 (482)	159 ± NR	Each subject was studied in randomly ordered 6-wk periods. They consumed cholesterol from eggs or removal of most cholesterol (~23 mg). Both diets replaced animal protein with textured vegetable protein. All meals were consumed in a metabolic ward.	TC, LDL, HDL, TG, LDL to HDL (6 wk)

(Continued)

TABLE 2 (*Continued*)

Lead author, year (country) (ref)	Study design	Mean age (range), y	Number analyzed (% male)	Dietary cholesterol dose[2] (difference)	Mean ± SD baseline TC, mg/dL	Intervention description	Outcomes (duration)
Kehoe, 1995[4] (United States) (54)	RCT (crossover)	NR (22–31)	28 (100)	800 vs. 125 (675)	See study	Subjects consumed an AHA step 1 diet at baseline with no egg yolks. Subjects were fed 1, 2, or 4 egg yolks and added fat to keep the diets comparable and blinded. Lunches and dinners were consumed in a monitored cafeteria, and subjects were given breakfast and snacks.	TC, LDL, HDL (8 wk)
Fielding, 1995 (United States) (39)	nRCT (parallel)	29[3] (20–35)	84 (100)	619 vs. 189 (430)	High dose: 167 ± 25; low dose: 162 ± 26	2-wk run-in, with 200 mg cholesterol/d. Experimental diets were isocaloric, fed in the research center, and only varied by dietary cholesterol.	TC, TG (4 wk)
Ginsberg, 1995 (United States) (44)	RCT (crossover)	24 (22–31)	13 (0)	770 vs. 108 (662)	157 ± 13	All subjects consumed a baseline step 1 diet with 125 mg cholesterol/d. Diets only differed by cholesterol content, with either eggs or egg substitutes added.	TC, LDL, HDL, TG (8 wk)
Ginsberg, 1994 (United States) (43)	RCT (crossover)	24 (22–31)	20 (100)	858 vs. 128 (730)	169 ± 18	3-wk run-in consuming average American diet (500 mg/d). Subjects either stayed on the American diet or switched to AHA step 1 diet.	TC, LDL, HDL, TG (8 wk)
Vorster, 1992[5] (South Africa) (56)	RCT (parallel)	18 (18–19)	44 (100)	556 vs. 800 (244)	NR	Subjects consumed 3 eggs for a 2-mo run-in. Group 1 continued a 3-egg/d intervention, group 2 ate 7 eggs/d, and group 3 ate 14 eggs/d. All 3 main meals were eaten in the university dining hall cafeteria, and fat and oil in recipes were obtained from the kitchen. The eggs were given at breakfast.	TC, LDL, HDL, TG, VLDL (12 wk)
Johnson, 1990 (United States) (48)	RCT (crossover)	27 (23–40)	10 (100)	600 vs. 200 (400)	169 ± 29	All foods were prepared in the clinical research center. Each subject underwent each intervention for 4 wk. The subjects consumed either a 600-mg/d diet with eggs or 200-mg/d diet with egg substitute. The diets were otherwise identical.	TC, LDL, HDL, TG, LDL to HDL (4 wk)
Clifton, 1990 (Australia) (38)	RCT (crossover)	42 (NR)	11 (73)	866 vs. 185 (681)	173 ± 25	4-wk run-in, with 180 mg cholesterol/d. Liquid supplements that contained either egg yolk or cholesterol-free fat mixture. Food records supervision to maintain equal nutrient composition.	TC, LDL, HDL, TG, VLDL, LDL to HDL

(Continued)

TABLE 2 (*Continued*)

Lead author, year (country) (ref)	Study design	Mean age (range), y	Number analyzed (% male)	Dietary cholesterol dose[2] (difference)	Mean ± SD baseline TC, mg/dL	Intervention description	Outcomes (duration)
Kestin, 1989 (Australia) (49)	RCT (crossover)	46 (NR)	10[6] (100)	711 vs. 192 (519)	255 ± 73	All subjects were given a high-fat diet for 4 wk to establish baseline plasma concentrations. Subjects either continued to consume the high-fat diet or switched to a modified-fat diet. Within each arm, subjects consumed an egg yolk supplement or egg substitute.	TC, LDL (4 wk)
Bowman, 1988 (United States) (37)	RCT (parallel)	22 (19–28)	19 (100)	501 vs. 207 (294)	High dose: 166 ± 2; low dose: 164 ± 2	2-wk run-in, with usual fat and usual cholesterol similar to an American diet (500 mg/d). Experimental diets were isocaloric and fed in the metabolic ward, only varying by dietary cholesterol.	TC, LDL, HDL, TG (5 wk)
Flynn, 1986 (United States) (42)	nRCT (crossover)	53 (34–68)	70 (77)	938 vs. 256 (682)	Group A/high dose: 189 ± 33; group B/high dose: 214 ± 56; group A/low dose: 185 ± 38; group B/low dose: 188 ± 40	One period with 3 eggs and one period with no eggs. Subjects ate free-living, self-selected customary diets with restrictions only to eggs, including baked goods containing eggs.	TC, TG (22 wk)[7]
Quig, 1983 (United States) (51)	RCT (parallel)	23 (20–28)	24 (100)	1400 vs. 400 (1000)	High dose: 170 ± 10; low dose: 186 ± 14	Subjects were assigned to moderate- (400 mg/g) or high- (1400 mg/d) cholesterol diets, which were part of 4-d menu cycles and were identical in nutrient composition. The extra cholesterol was provided by 4 whole medium eggs.	TC, LDL, HDL, TG (4 wk)
Flaim, 1981 (United States) (40)	RCT (crossover)	23 (20–30)	23 (100)	1415 vs. 415 (1000)	High dose: 166 ± 9; low dose: 170 ± 8	High-cholesterol diet was similar to control diet with addition of 4 eggs. Control diet was typical of common American diet with only nonegg cholesterol sources. All meals fed in a metabolic ward.	TC, LDL, HDL, TG, VLDL (4 wk)
Flynn, 1979 (United States) (41)	nRCT (crossover)	46 (32–32)	116 (100)	800 vs. 260 (540)	Group 1: 214 ± 35; group 2: 203 ± 39	Experimental diet contained 2 whole eggs, and the control diet contained no eggs with restrictions on baked goods/foods containing eggs. Subjects ate customary diets throughout the study.	TC, HDL, TG (24 wk)

[1]AHA, American Heart Association; NCEP, National Cholesterol Education Program; NR, not reported; nRCT, nonrandomized controlled trial; RCT, randomized controlled trial; ref, reference; TC, total cholesterol; TG, triglycerides.

[2]When subgroups had slightly different cholesterol doses, the values were averaged for reporting. Doses are exact in the meta-analysis.

[3]Mean value calculated.

[4]Study was used for qualitative analysis only because results could not be combined with other studies.

[5]Group with 3 eggs was not included in the analysis because baseline values were 2 mo earlier than 7- and 14-egg groups.

[6]Study included 25 subjects, but only 10 subjects met inclusion criteria.

[7]One group participated for 10 wk, the other for 12 wk.

TABLE 3
Effect sizes for the association between dietary cholesterol and blood cholesterol concentrations among intervention studies

Analysis subgroup	Total cholesterol	LDL cholesterol	HDL cholesterol	Triglycerides	VLDL	LDL to HDL ratio
Overall						
Net change (95% CI), mg/dL	11.2 (6.4, 15.9)	6.7 (1.7, 11.7)	3.2 (0.9, 9.7)	0.1 (−6.8, 7.0)	0.6 (−2.2, 3.3)	0.2 (0.0, 0.3)
I^2, P value	75%, <0.001	65%, <0.001	70%, <0.001	73%, <0.001	69%, 0.04	0%, 0.70
Overall (no extreme values ≥1000 or <1 mg/d)						
Net change (95% CI), mg/dL	11.7 (8.0, 15.4)	7.5 (4.5, 10.5)	3.1 (0.3, 5.9)	−0.3 (−7.7, 7.2)	—	—
I^2, P value	45%, 0.01	0%, 0.45	70%, <0.001	71%, <0.001		
Randomized vs. nonrandomized						
Randomized						
Net change (95% CI), mg/dL	7.4 (1.8, 12.9)	5.5 (0.3, 10.7)	2.8 (0.6, 5.1)	−2.3 (−12.4, 7.9)	—	—
I^2, P value	75%, <0.001	61%, <0.001	52%, 0.01	85%, <0.001		
Nonrandomized						
Net change (95% CI), mg/dL	18.1 (9.5, 26.7)	12.8 (−3.7, 29.3)	3.7 (−2.2, 9.7)	3.8 (−3.3, 10.8)	—	—
I^2, P value	66%, 0.003	80%, 0.008	87%, <0.001	0%, 0.91		
Randomized vs. nonrandomized (no extreme values ≥1000 or <1 mg/d)						
Randomized						
Net change (95% CI), mg/dL	8.1 (6.1, 10.2)	6.9 (3.6, 10.2)	2.0 (−0.42, 4.4)	−3.3 (−14.9, 8.2)	—	—
I^2, P value	0%, 0.94	0%, 0.98	22%, 0.25	83%, <0.001		
Nonrandomized						
Net change (95% CI), mg/dL	18.1 (9.5, 26.7)	12.8 (−3.7, 29.3)	3.7 (−2.2, 9.6)	3.8 (−3.3, 10.8)	—	—
I^2, P value	66%, 0.003	80%, 0.01	87%, <0.001	0%, 0.91		
Intervention strata						
>900 mg/d						
Net change (95% CI), mg/dL	13.0 (−3.1, 29.1)	1.6 (−18.8, 22.0)	4.5 (−3.2, 12.1)	3.0 (−3.2, 9.2)	—	—
I^2, P value	94%, <0.001	89%, <0.001	93%, <0.001	0%, 0.59		
650–900 mg/d						
Net change (95% CI), mg/dL	10.7 (5.4,15.9)	8.7 (3.8, 13.5)	2.7 (0.7, 4.7)	2.3 (−4.3, 8.9)	—	—
I^2, P value	34%, 0.11	14%, 0.31	27%, 0.20	0%, 0.96		
<650 mg/d						
Net change (95% CI), mg/dL	12.1 (6.0, 18.2)	6.7 (2.7, 10.7)	1.0 (−2.1, 4.1)	−2.4 (−19.0, 14.3)	—	—
I^2, P value	40%, 0.14	0%, 0.74	0%, 0.43	93%, <0.001		
Intervention strata (no extreme values ≥1000 or <1 mg/d)						
>900 mg/d						
Net change (95% CI), mg/dL	19.3 (2.3, 36.3)	—	—	—	—	—
I^2, P value	75%, 0.02					
650–900 mg/d						
Net change (95% CI), mg/dL	10.7 (5.4, 15.9)	—	—	—	—	—
I^2, P value	34%, 0.11					
<650 mg/d						
Net change (95% CI), mg/dL	13.4 (4.9, 21.9)	—	—	—	—	—
I^2, P value	63%, 0.05					
Control strata						
>200 mg/d						
Net change (95% CI), mg/dL	11.6 (2.0, 21.0)	3.6 (−5.6, 12.7)	3.7 (−0.5, 8.0)	−0.2 (−10.8,10.5)	—	—
I^2, P value	83%, <0.001	76, <0.001	84%, <0.001	81%, <0.001		
≤200 mg/d						
Net change (95% CI), mg/dL	11.1 (6.8, 15.4)	8.8 (4.5, 13.2)	2.5 (0.8, 4.2)	0.6 (−3.8, 5.1)	—	—
I^2, P value	42%, 0.06	21%, 0.25	0%, 0.65	0%, <0.001		

change was 5.5 mg/dL (95% CI: 0.3, 10.7) with significant heterogeneity (I^2 = 61%, P < 0.001). Among the 13 randomized trials, 8 were rated with a low risk of bias (37, 38, 42–45, 49, 56), and the remaining 5 were rated medium risk of bias (12, 40, 49–51). The nonrandomized studies showed a nonsignificant increase in LDL cholesterol of 12.8 mg/dL (95% CI: −3.7, 29.3) with significant heterogeneity (I^2 = 80%, P = 0.008). Both of the nonrandomized trials were assigned a medium risk of bias (8, 47).

When studies measuring LDL cholesterol were stratified by intervention dose of dietary cholesterol, studies with an intervention dose <650 mg/d showed a significant increase in serum LDL cholesterol concentration (net change: 6.7; 95% CI: 2.7, 10.7) with no heterogeneity (I^2 = 0%, P = 0.74; **Figure 3**). Similarly, studies with an intervention dose from 650 to 900 mg/d showed a significant increase in LDL cholesterol (net change: 8.7 mg/dL; 95% CI: 3.8, 13.5) with no significant heterogeneity

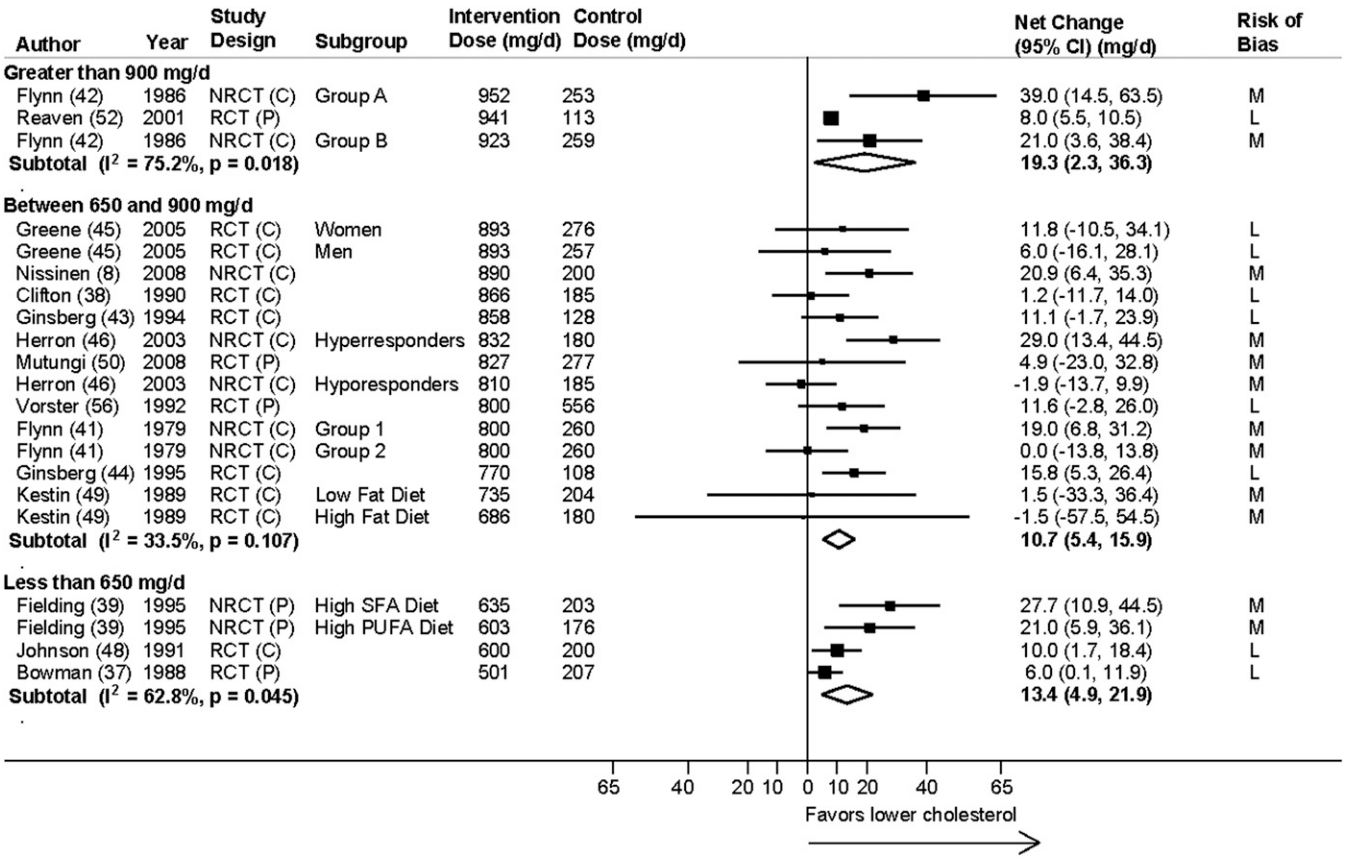

FIGURE 2 Meta-analysis examining the effect of dietary cholesterol on total cholesterol concentration by strata of intervention dose with no extreme values. Studies with intervention doses ≥1000 mg/d or <1 mg/d were considered extreme dose values. Black squares represent point estimates for individual studies, and the width of the line extending from each square represents the 95% CI. The diamond represents the overall effect, and the width of the diamond represents the 95% CI of the summary net change. I^2 is an indicator of between-comparison heterogeneity. Heterogeneity was significant at $P < 0.10$. L, low risk of bias; M, medium risk of bias; NRCT (C), crossover, nonrandomized controlled trial; NRCT (P), parallel, nonrandomized controlled trial; RCT (C), crossover, randomized controlled trial; RCT (P), parallel, randomized controlled trial.

(I^2 = 14%, P = 0.31). In studies with an intervention dose >900 mg/d, the effect of dietary cholesterol on LDL cholesterol was no longer significant (net change: 1.6; 95% CI: −18.8, 22.0) with statistically significant heterogeneity (I^2 = 89%, $P < 0.001$). When nonrandomized studies were removed from analysis, the effect of dietary cholesterol on LDL cholesterol remained the same, and heterogeneity further decreased in studies with an intervention dose from 650 to 900 mg/d (data not shown).

Additional analysis was performed to separate studies on LDL cholesterol by the control dose of dietary cholesterol. The effect of dietary cholesterol on LDL cholesterol in studies with a control dose >200 mg/d was not significant (net change: 3.6; 95% CI: −5.6, 12.7), and heterogeneity was significant (I^2 = 76%, $P <$ 0.001). In studies with a control dose of dietary cholesterol ≤200 mg/d, LDL cholesterol statistically significantly increased 8.8 mg/dL (95% CI: 4.5, 13.2) with no statistically significant heterogeneity (I^2 = 21%, P = 0.25).

HDL cholesterol

Fourteen unique trials among 15 studies reported the effect of dietary cholesterol interventions on serum concentration of HDL cholesterol (8, 37, 38, 40, 42–48, 50, 51, 56). Of the 14 trials, 11 were randomized (37, 38, 40, 43–45, 47, 48, 50, 51, 56) and 3 were nonrandomized trials (8, 42, 46).

Meta-analysis of trials measuring HDL cholesterol showed a significant increase in serum HDL cholesterol (net change: 3.2 mg/dL; 95% CI: 0.9, 9.7) with significant heterogeneity (I^2 = 70%, $P < 0.001$; Table 3). When stratified by randomization, the effect of dietary cholesterol on HDL cholesterol concentration remained significant in randomized trials (net change: 2.8; 95% CI: 0.6, 5.1) with significant heterogeneity (I^2 = 52%, P = 0.01). Among the 11 randomized trials, 7 were rated with a low risk of bias (37, 38, 44, 45, 47, 51, 56), and the remaining 4 were rated a medium risk of bias (40, 47, 51, 53). In nonrandomized trials, the effect of dietary cholesterol on HDL cholesterol was no longer statistically significant (net change: 3.7; 95% CI: −2.2, 9.7) with significant heterogeneity (I^2 = 87%, $P < 0.001$). All 3 nonrandomized trials were assigned a medium risk of bias (8, 42, 46).

When studies measuring HDL cholesterol were stratified by intervention dose of dietary cholesterol, studies with a dose <650 mg/d did not show a significant effect of dietary cholesterol on HDL cholesterol (net change: 1.0 mg/dL; 95% CI: −2.1, 4.1) with no heterogeneity (I^2 = 0.0%, P = 0.44; **Figure 4**). In studies with an intervention dose from 650 to 900 mg/d, there was a significant effect of dietary cholesterol on HDL concentration (net change: 2.7 mg/dL; 95% CI: 0.7, 4.7), and heterogeneity was not significant (I^2 = 27%, P = 0.20). In studies with an intervention dose >900 mg/d, the effect of dietary cholesterol on HDL cholesterol concentration was not significant (net change:

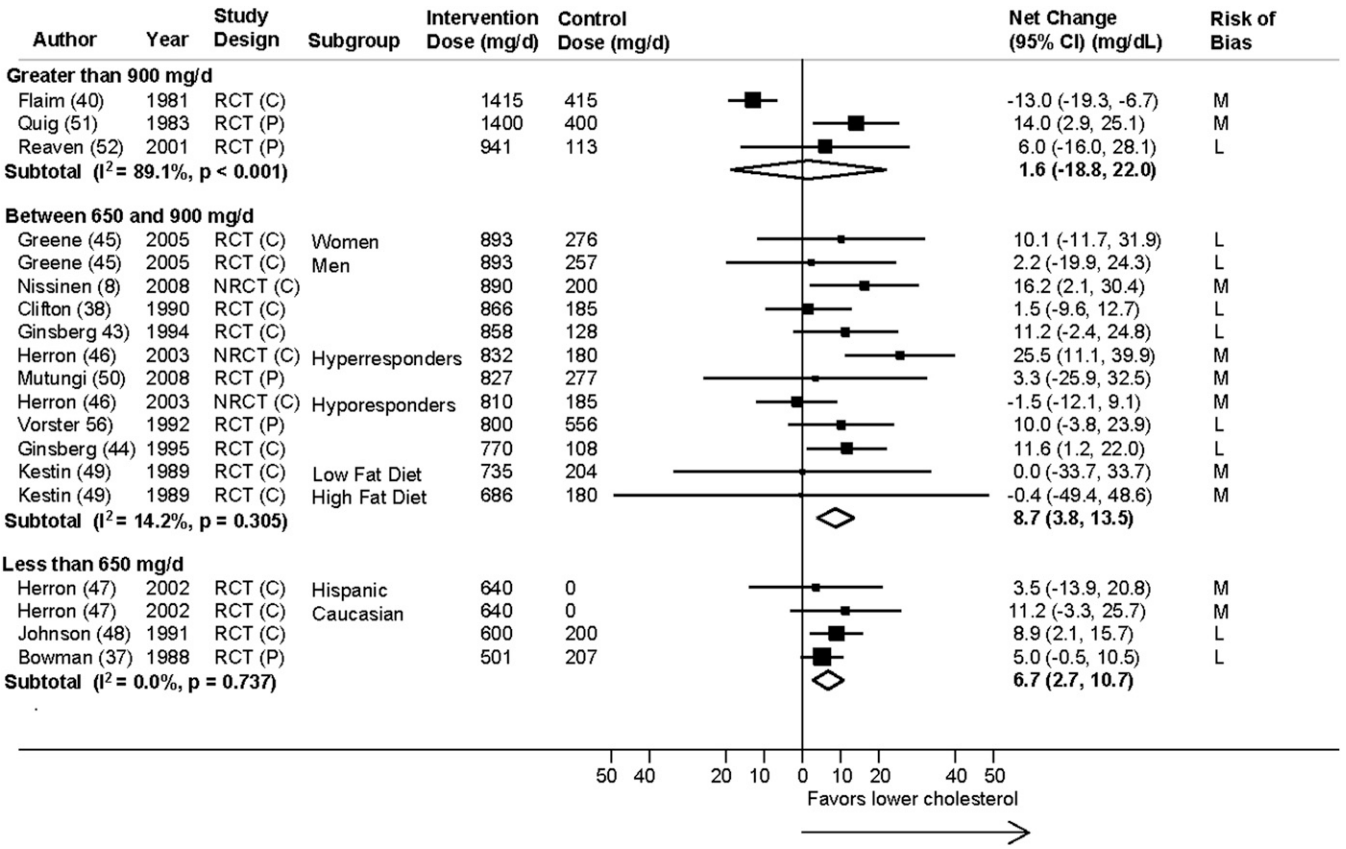

FIGURE 3 Meta-analysis examining the effect of dietary cholesterol on LDL cholesterol concentration by strata of intervention dose. Black squares represent point estimates for individual studies, and the width of the line extending from each square represents the 95% CI. The diamond represents the overall effect, and the width of the diamond represents the 95% CI of the summary net change. I^2 is an indicator of between-comparison heterogeneity. Heterogeneity was significant at $P < 0.10$. L, low risk of bias; M, medium risk of bias; NRCT (C), crossover, nonrandomized controlled trial; RCT (C), crossover, randomized controlled trial; RCT (P), parallel, randomized controlled trial.

4.5 mg/dL; 95% CI: −3.2, 12.1) with statistically significant heterogeneity (I^2 = 93%, $P < 0.001$). When nonrandomized studies were removed from analysis, the effect of dietary cholesterol on HDL cholesterol remained the same in studies with an intervention dietary cholesterol dose ≤900 mg/d (data not shown). Randomized studies with a dose >900 mg/d could not be combined because only 2 randomized trials (40, 51) reported the effect of dietary cholesterol on HDL cholesterol concentration when cholesterol dose was >900 mg/d (data not shown).

We performed additional analysis to separate studies on HDL cholesterol by the control dose of dietary cholesterol. The effect of dietary cholesterol on HDL cholesterol in studies with a control dose >200 mg/d was not significant (net change: 3.7; 95% CI: −0.5, 8.0), with significant heterogeneity (I^2 = 84%, $P < 0.001$). In studies with a control dose of dietary cholesterol ≤200 mg/d, higher dietary cholesterol statistically significantly increased HDL cholesterol concentration by 2.5 mg/dL (95% CI: 0.8, 4.2), with no heterogeneity (I^2 = 0%, P = 0.65).

Triglycerides

Of the 13 trials that measured serum triglycerides (8, 37–47, 56), 7 trials were randomized (37, 38, 40, 43–45, 48) and 6 were nonrandomized (8, 39, 41, 42, 46, 56). In overall analysis and in stratification by randomized design, dietary cholesterol did not statistically significantly change serum triglycerides. Overall, triglycerides decreased by 0.1 mg/dL (95% CI: −6.8, 7.0) with significant heterogeneity (I^2 = 73%, $P < 0.001$; Table 3). Among randomized studies, the change in triglycerides was not significant by −2.3 mg/dL (95% CI: −12.4, 7.9), with significant heterogeneity (I^2 = 85%, $P < 0.001$). Among nonrandomized studies, the change in triglycerides was not significant (net change: 3.8 mg/dL; 95% CI: −3.3, 10.8), with no heterogeneity (I^2 = 0%, P = 0.91). Among the randomized studies, 7 were rated with a low risk of bias (37, 38, 44, 45, 48, 51, 56), and one was rated a medium risk of bias (40). All nonrandomized studies were rated a medium risk of bias (8, 39, 41, 42, 46). The effect of dietary cholesterol on triglycerides remained nonsignificant when studies were stratified by intervention dose and control dose of dietary cholesterol.

VLDL cholesterol

Three studies reported the effect of dietary cholesterol on VLDL cholesterol concentrations (38, 40, 56). All 3 studies were randomized. Meta-analysis showed no statistically significant increase in VLDL cholesterol concentration when comparing intervention with control doses of dietary cholesterol (net change: 0.56 mg/dL; 95% CI: −2.2, 3.3; Table 3) with statistically significant heterogeneity (I^2 = 69%, P = 0.04). Two studies were rated a low risk of bias (38, 56) and one study was rated a medium risk of bias (40). There were too few studies reporting VLDL concentration for further subgroup analysis.

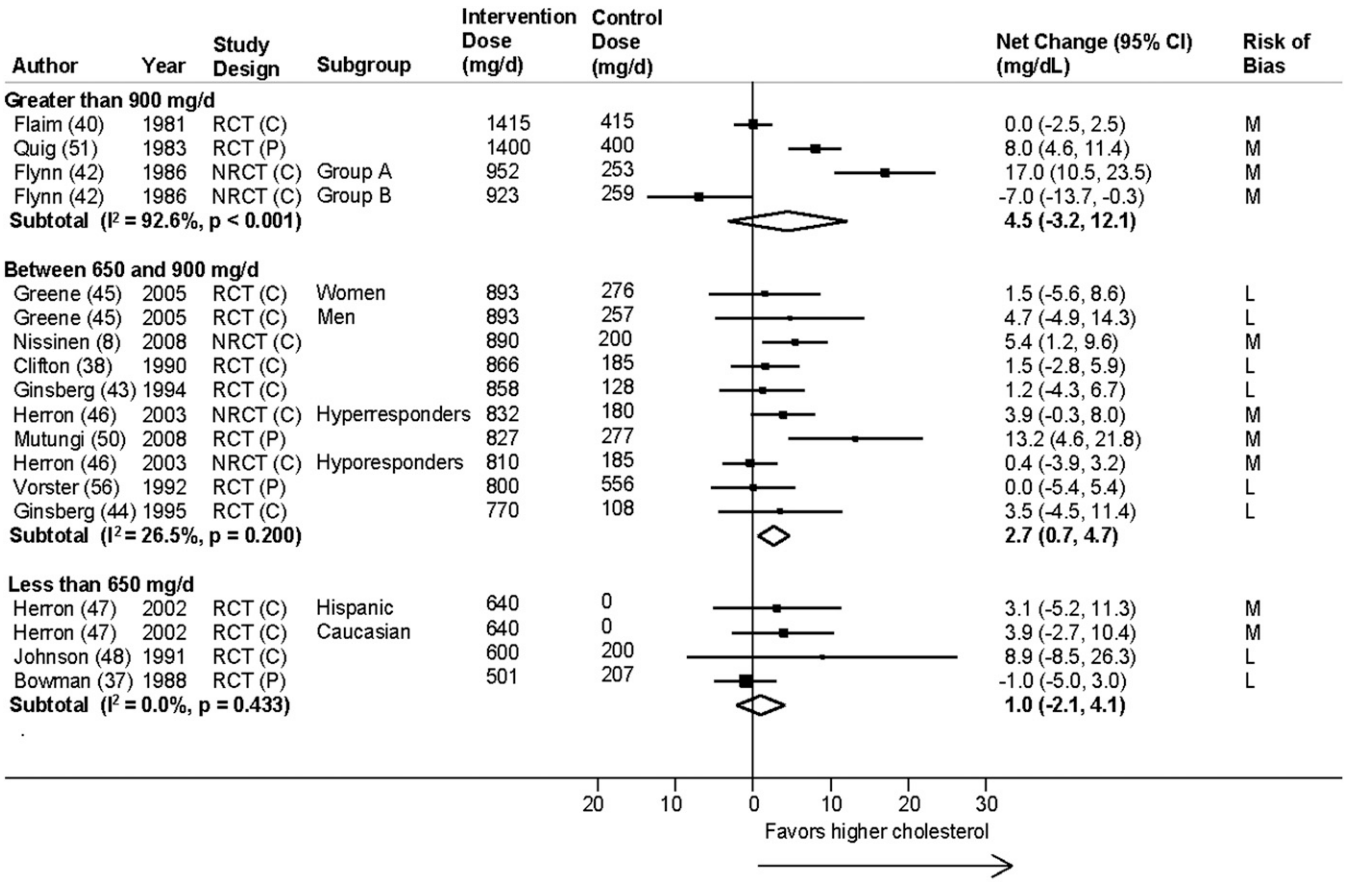

FIGURE 4 Meta-analysis examining the effect of dietary cholesterol on HDL cholesterol concentration by strata of intervention dose. Black squares represent point estimates for individual studies, and the width of the line extending from each square represents the 95% CI. The diamond represents the overall effect, and the width of the diamond represents the 95% CI of the summary net change. I^2 is an indicator of between-comparison heterogeneity. Heterogeneity was statistically significant at $P < 0.10$. L, low risk of bias; M, medium risk of bias; NRCT (C), crossover, nonrandomized controlled trial; RCT (C), crossover, randomized controlled trial; RCT (P), parallel, randomized controlled trial.

LDL to HDL cholesterol ratio

Seven intervention studies reported the effect of dietary cholesterol on the ratio of LDL to HDL cholesterol (38, 45–48, 50, 55). Four studies provided complete data and were included in meta-analysis (45–47, 50). Overall analysis showed a statistically significant increase in the LDL to HDL cholesterol ratio of 0.17 (95% CI: 0.01, 0.32; Table 3 and **Figure 5**) with no heterogeneity ($I^2 = 0\%$, $P = 0.70$). There were too few studies to perform subgroup analysis.

Subgroup and dose-response analyses

Meta-regression found no linear relations between net change in total, LDL, or HDL cholesterol or triglycerides and intervention dose of dietary cholesterol. We also investigated the linear relation between serum cholesterol and difference in dose (the difference between intervention and control dose), because the studies present a wide range of intervention and control dose comparisons. No linear relations were found between total cholesterol, LDL, HDL, or triglycerides and dose difference. When extreme dose values were removed and data were stratified by age, we found nonsignificant linear trends when studies were stratified by a mean age of 40 y. Age-stratified meta-regression plots and β coefficients for total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides are available in **Supplemental Figures 5–8**. Nonlinear regression analysis of all studies did not fit models for total cholesterol or LDL outcomes. For studies reporting HDL outcomes, when recent studies were included (1990–2008, $n = 12$), a model fit the second-order polynomial for the association between dietary cholesterol and HDL cholesterol [% Change in HDL = 37.6 – 0.012 (dose) – 0.00004 (dose^2)]. Most studies reporting HDL outcomes showed an increase in HDL cholesterol with increased doses of dietary cholesterol (**Figure 6**).

Additional subgroup analysis was performed on age and sex. There were no changes in the effect of dietary cholesterol on any serum cholesterol outcomes when studies were stratified by those older or younger than 40 y of age or when stratified by males only, females only, or both sexes combined. Too few studies analyzed females only or both sexes combined to perform analysis on sex within dose strata.

Sensitivity analysis

We performed 3 sets of sensitivity analyses to better understand the heterogeneity across studies. We preformed sensitivity analyses for one study that presented findings based on subgroups (47), for studies with extreme values of dietary cholesterol intervention doses, and for nonrandomized trials. One study presented 2 sets of subgroup analyses, one by Caucasian and Hispanic ethnicities and

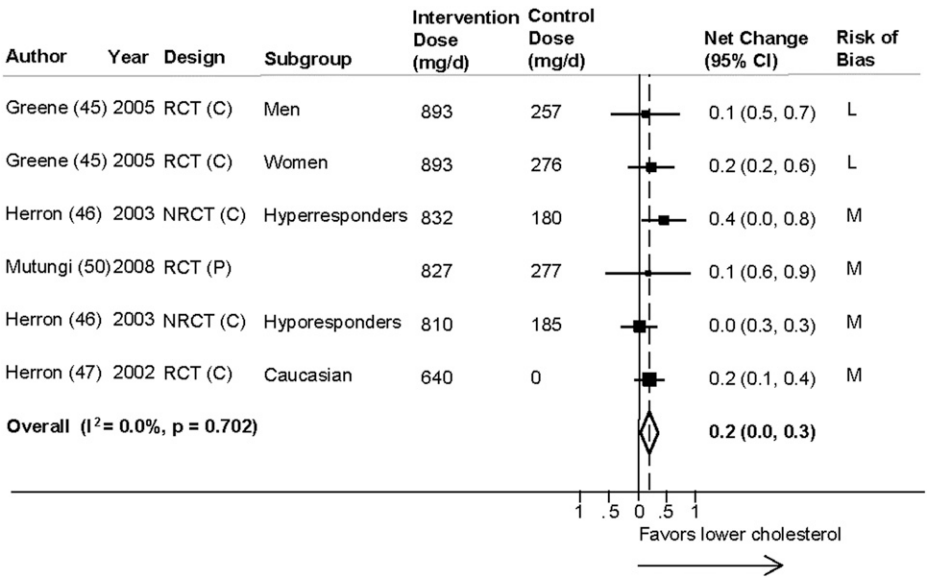

FIGURE 5 Meta-analysis examining the effect of dietary cholesterol on the LDL to HDL ratio. Net change indicates change in the LDL to HDL ratio. L, low risk of bias; M, medium risk of bias; NRCT (C), crossover, nonrandomized controlled trial; RCT (C), crossover, randomized controlled trial; RCT (P), parallel, randomized controlled trial.

another by hyper- and hyporesponders based on serum cholesterol concentrations after randomization (47). We used the ethnicity subgroups in the main analyses to retain study randomization but found no difference in overall findings with the hyper- and hyporesponder subgroups.

We also performed a meta-analysis excluding studies by using extreme dietary cholesterol intervention doses (40, 47, 51) (≥1000 mg/d or a control dose of <1 mg/d; Table 3). The removal of extreme doses further increased total cholesterol effect by 1 mg/dL (from 7.4 to 8.1 mg/dL) for randomized studies, and the CI became tighter. There were no statistically significant changes in the nonrandomized studies. We found a similar increase in LDL cholesterol in the randomized studies of 1 mg/dL (from 5.5 to 6.9 mg/dL). The results became statistically significant (net change: 6.7; 95% CI: 3.3, 10.1), and heterogeneity was reduced (I^2 = 0%, P = 0.970). On removal of extreme values, the increase in HDL cholesterol lessened (from 2.8 to 2.4 mg/dL), and the heterogeneity was no longer statistically significant (I^2 = 29%, P = 0.20). Removing nonrandomized trials from across analyses of serum cholesterol outcomes did not affect the overall conclusions (data not shown).

Publication bias

Funnel plots of all studies reporting total, LDL, and HDL cholesterol outcomes indicate a potential for missing studies with an effect of decreasing lipid concentrations after intake of dietary cholesterol (net difference <0). Egger's test suggests no small study effect. Funnel plots and Egger test results can be found in **Supplemental Figures 9–14**. Publication bias was not assessed for cohort studies because <10 studies were available for each outcome.

DISCUSSION

In the studies reviewed, higher intake of cholesterol was not associated with an increased risk of incident CVD. However, the evidence from cohort studies was sparse, limiting our ability to perform meta-analyses for most CVD outcomes. In intervention trials, there was a statistically significant effect of cholesterol intake on total cholesterol, LDL cholesterol, and the LDL to HDL cholesterol ratio. The increases in total and LDL cholesterol were no longer significant in studies with intervention doses >900 mg/d. HDL concentration also increased with higher cholesterol intake, particularly in randomized studies and studies with intervention doses between 650 and 900 mg/d. Because data on the LDL to HDL cholesterol ratio with increased dietary cholesterol were limited, further studies are necessary to help interpret the change in CVD risk associated with higher cholesterol intakes. There was no effect on VLDL cholesterol or triglycerides with higher doses of dietary cholesterol.

There is a plausible mechanism for the effect of dietary cholesterol on serum lipid concentrations. Higher total cholesterol,

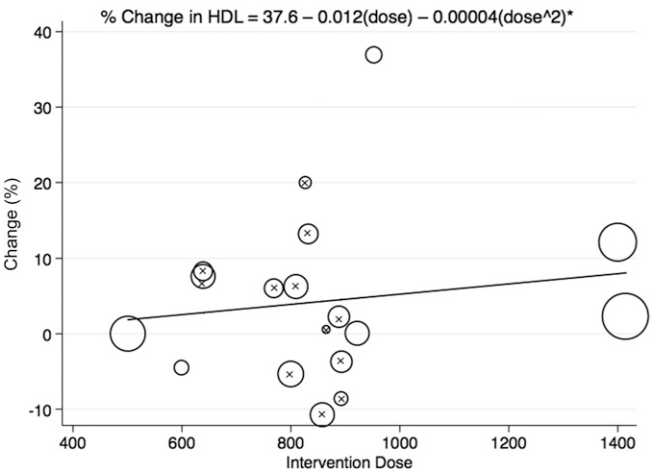

FIGURE 6 Dose-response relation between change in HDL cholesterol (%) and dietary cholesterol for studies reporting HDL outcomes (n = 18). *Of 18 studies reporting HDL cholesterol outcome, a polynomial model was derived from 12 recent studies published between 1990 and 2008.

LDL cholesterol, and triglycerides, as well as lower HDL cholesterol, are known risk factors for CVD (1, 57). Given that cholesterol is synthesized in the body, there is compensation for absorption of additional dietary cholesterol by reducing cholesterol synthesis (58). Major dietary sources of cholesterol include egg yolks, butter, fish, shrimp, cheese, beef, pork, and poultry. Cholesterol is mostly endogenous in origin and is only one of the constituents of end-stage atherosclerosis. The relation between dietary cholesterol and serum cholesterol has been estimated to be linear with cholesterol intake up to 600 mg/d. Studies have reported a nonlinear relation for intakes of cholesterol >600 mg/d, with little effect on serum lipid concentration in most people (59). The findings from our meta-analysis indicate that increases in serum cholesterol are no longer statistically significant when dietary cholesterol interventions exceed 900 mg/d, which is consistent with previous observations showing a plateau in serum cholesterol concentrations when dietary cholesterol increases (60).

In contrast to a prior meta-analysis (61), we did not find a linear relation between cholesterol intake and serum lipids. The contrast in findings from the prior meta-analysis and the results of this systematic review could be explained by a variation in criteria for study inclusion. For example, our study excluded trials with <5 subjects per arm, <4 wk, and hypercholesterolemic populations, whereas Weggemans et al. (61) included studies with <10 subjects per arm, excluded studies <2 wk, and included hypercholesterolemic populations. This contrast in eligibility criteria led to a different set of included and excluded studies in the prior meta-analysis and this systematic review.

There are several possible explanations for the association between the dietary cholesterol intake and CVD outcomes in some studies (26, 32) and not in others (21, 25, 27–29). First, the presence of association between cholesterol intake and CVD outcomes may be confounded by a variety of dietary factors, which may exaggerate the true relation. Risk of CVD has been reported to be related to increased consumption of saturated fatty acids and percentage of calories from fat (27, 62, 63), which are positively associated with cholesterol intake. Likewise, risk of CVD has negative associations with fiber intake and vegetable protein, which are inversely correlated with cholesterol intake (64–66). The reviewed studies rarely adjusted for these key dietary variables in their multivariable analyses. Second, there is less diagnostic certainty in the measurement of clinical endpoints using death certificates or International Classification of Diseases codes for assessment of these outcomes (67), which may lead to an attenuation of a true relation in some studies. Third, cohort studies used a single baseline measurement across years of follow-up (i.e., making the assumption that diet is unchanging over time), resulting in bias, often referred to as regression dilution bias (68). That is, any dietary changes occurring during follow-up are not captured, and thus misclassification of cholesterol intake will increase over time, resulting in possible attenuation of relations between dietary cholesterol and CVD.

In this review, we identified areas of further exploration on dietary cholesterol and CVD risk. In the included trials, healthy adults consumed high doses of dietary cholesterol, mostly prepared from eggs. The intervention doses administered were typically greater than average American intakes of cholesterol. The mean intake for adults in the United States is 350 mg/d for men and 240 mg/d for women (2), whereas the doses in the included trials ranged from 500 to 1400 mg/d. Further trials are warranted to examine the effect of cholesterol intakes between 300 and 500 mg/d on serum lipids in healthy populations to better understand the possible effects of typical cholesterol intakes at the population level.

In addition, eligible studies reported limited subgroup data to account for individual variation in response to dietary cholesterol (69). This variation can partly be explained by factors including, ethnicity, hormonal status, obesity, lipoprotein disorders, and genetic predisposition (7, 70). Few studies in our review considered separate sex, age, ethnicity, and hyper- or hyporesponding subgroups. Further studies are warranted to examine the role of individual variation in serum lipid concentration response to increasing dietary cholesterol.

The limitations of this review reflect, to a large extent, the limitations of the data available in primary studies. In the observational studies, the outcome was ascertained differently across studies by self-report, medical record examination, or death certificates. A positive self-report is generally quite accurate in large epidemiologic studies. However, dietary exposures are more prone to misclassification. In the observational studies included in the review, cholesterol intake was typically ascertained only once at baseline, but exposure ascertainment once at baseline may not truly reflect long-term intake status. There was also substantial heterogeneity among studies. Studies evaluated a variety of CVD outcomes, used a mixture of metrics to measure disease risk, and used a range of potential confounders in multivariable analysis. Importantly, only a handful of studies adjusted for potentially confounding dietary variables such as fiber, energy, and saturated fat. Finally, study participants were mostly males, and race-ethnicity information was rarely reported, thus limiting the applicability of the reviewed evidence to other racial groups and women. In the intervention studies, the studies reviewed were also heterogeneous, with varying randomization and design schemes, a wide variety of intervention and control doses of dietary cholesterol, and different populations and subgroups of interest. We accounted for design and dose differences in subgroup analysis; however, there were too few studies to define the effect of dietary cholesterol on blood cholesterol concentrations within population subgroups.

The role of LDL and HDL subparticles has not been examined in this review. Emerging data from other studies suggest that large LDL and large HDL associated with dietary cholesterol are less atherogenic lipoproteins and offer an increased protection against atherosclerosis (71, 72). Large HDL has been related to efficient reverse cholesterol transport and improved metabolic health. Further studies are needed to assess the effects of dietary cholesterol on lipoprotein subparticles.

In conclusion, the effect of dietary cholesterol on incident CAD and serum cholesterol outcomes remains unclear. Intervention trials showed a statistically significant increase in total, LDL, and HDL cholesterol when comparing intervention doses of 500–900 mg/d dietary cholesterol with control doses. Lower intake of dietary cholesterol has been recommended by some to optimize clinical outcomes or prevent incident CAD; however, there is a lack of longitudinal data (observational or trials) to support such a recommendation. It is therefore imperative that longitudinal observational studies are conducted with frequent exposure ascertainment and appropriate control for potential dietary confounders. Additional long-term trials should be

conducted to examine dietary intake of cholesterol between 300 and 500 mg/d to test the potential role of typical dietary cholesterol intakes on clinical outcomes.

We thank Dr. Elias Zintzaras for assistance with statistical analysis. SB, GR, RV, and EJJ: conceived and designed the study; SB, GR, RV, and EJJ: analyzed and interpreted the data; SB, GR, and EJJ: drafted the article; RV, PFJ, and EJJ: critically revised the article for important intellectual content; PFJ and EJJ: provided final approval of the article; SB, GR, RV, and EJJ: collected and assembled the data. No authors declared a conflict of interest related to this study.

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